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REVIEW ARTICLE

Oxygen toxicity, oxygen radicals, transition metals and disease

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Introduction

The frequency with which oxygen is used in clinical medicine must not blind us to the fact that it has many toxic effects. O_2 supplied at concentrations greater than those in normal air has long been known to damage plants, animals and aerobic bacteria such as *Escherichia coli* (for reviews see Morris, 1976 and Balentine, 1982). Plots of the logarithm of survival time against logarithm of the oxygen pressure have shown inverse, approximately linear, relationships for protozoa, mice, fish, rats, rabbits and insects. Indeed, there is considerable evidence that even 21% O_2 has slowly-manifested damaging effects. The effects observed vary considerably with the type of organism used, its age, physiological state and diet, such as the presence in the diet of varying amounts of vitamins A, E and C, transition metals, antioxidants (now added to many animal and human foods) and polyunsaturated lipids.

In 1954, Rebecca Gershman and Daniel L. Gilbert proposed that many of the damaging effects of O_2 could be attributed to the formation of O_2 radicals (for a review see Gilbert, 1981). This hypothesis was developed by Fridovich (1975, 1978, 1983) into a superoxide theory of oxygen toxicity, which states that formation of the superoxide radical *in vivo* plays a major role in the toxic effects of oxygen. The purpose of this article is to explain what oxygen radicals are, how transition metals are involved in their formation and reactivity, and the role played by radicals and metals in some disease states.

Oxygen free-radicals

A free-radical may be defined as any species that has one or more unpaired electrons. This broad definition includes the hydrogen atom (one unpaired electron), most transition metals and the oxygen molecule itself. O_2 has two unpaired electrons, each located in a different π^* antibonding orbital (for a detailed explanation see Halliwell, 1981*b*). These two electrons have the same spin quantum number and so if O_2 attempts to oxidize another atom or molecule by accepting a pair of electrons from it, both new electrons must be of parallel spin so as to fit into the vacant spaces in the π^* orbitals. A pair of electrons in an atomic or molecular orbital would however have antiparallel spins (of $+\frac{1}{2}$ and $-\frac{1}{2}$), in accordance with Pauli's principle. This imposes a restriction on oxidations by O_2 which tends to make O_2 accept its electrons one at a time and slows its reaction with non-radical species (Fig: 1). Transition metals are found at the active site of many oxidases and oxygenases because their ability to accept and donate single electrons can overcome this spin restriction (Hill, 1981).

Abbreviations used: SOD, superoxide dismutase; DETAPAC, diethylenetriamine penta-acetic acid.

Singlet oxygen

Another way of increasing the reactivity of O_2 is to move one of the unpaired electrons in a way that alleviates the spin restriction. Two singlet states of O_2 exist (Fig. 1). Singlet $O_2{}^1\Delta g$, the most important in biological systems, has no unpaired electrons and is not a radical. Singlet $O_2{}^1\Sigma g^+$ usually decays to the $^1\Delta g$ state before it has time to react with anything. Excitation of O_2 to the $^1\Delta g$ state can be achieved when several biological pigments, such as chlorophylls, retinal, flavins or porphyrins, are illuminated in the presence of O_2 (Foote, 1982). Formation of singlet O_2 occurs *in vivo* in illuminated chloroplasts (Halliwell, 1981*a*) and in both the lens and retina of the mammalian eye (Zigler & Goosey, 1981; Katz *et al.*, 1982; Kirschfeld, 1982).

Superoxide

If a single electron is accepted by the ground-state O_2 molecule, it must enter one of the π^* antibonding orbitals. The product is superoxide radical, O_2^- (Fig. 1). (Remember that oxygen itself is a radical, however, with two unpaired electrons as compared with superoxide's one). O_2^- is formed in almost all aerobic cells (Fridovich, 1975, 1978; Halliwell, 1981*b*), one important source being the 'respiratory burst' of phagocytic cells when they contact foreign particles or immune complexes (for reviews see Babior, 1978*a*,*b*; Halliwell, 1982). Phagocytic cells known to produce O_2^- include neutrophils, monocytes, macrophages and eosinophils.

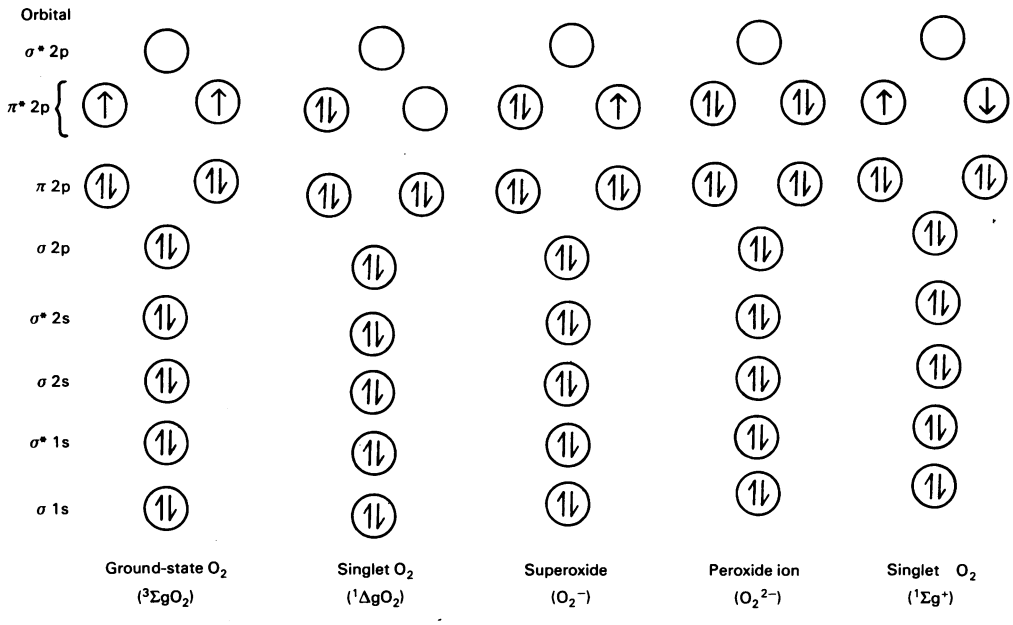

Fig. 1. *Bonding in the diatomic oxygen molecule*

Hydrogen peroxide

Addition of a second electron to O_2^- gives the peroxide ion, O_2^{2-}, which has no unpaired electrons and is not a radical. Any O_2^{2-} formed at physiological pH will immediately protonate to give hydrogen peroxide (H_2O_2) since the pK_a of H_2O_2 is very high. In aqueous solution, O_2^- undergoes the so-called dismutation reaction to form H_2O_2 and O_2. The overall reaction can be written

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \qquad (1)$$

although it is in fact the sum of several stages. The rate of dismutation is faster at acidic pH values.

Hydroxyl radical

Homolytic fission of the O–O bond in H_2O_2 produces two hydroxyl radicals, ·OH. Homolysis can be achieved by heat or ionising radiation. A simple mixture of H_2O_2 and an iron (II) salt also forms the ·OH radical, as was first observed by Fenton in 1894 (see Walling, 1982, for a review):

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^- \qquad (2)$$

Traces of Fe^{3+} can react further with H_2O_2:

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + O_2^- + H^+ \qquad (3)$$

and more reactions are possible:

$$\cdot OH + H_2O_2 \rightarrow H_2O + H^+ + O_2^- \qquad (4)$$

$$O_2^- + Fe^{3+} \rightarrow Fe^{2+} + O_2 \qquad (5)$$

$$\cdot OH + Fe^{2+} \rightarrow Fe^{3+} + OH^- \qquad (6)$$

Thus a simple mixture of an iron salt and H_2O_2 can provoke a whole series of radical reactions. The overall sum of these, unless some other reagent is added to intercept ·OH, is an iron-catalysed decomposition of H_2O_2:

$$2H_2O_2 \xrightarrow[\text{catalyst}]{\text{Fe salt}} 2H_2O + O_2 \qquad (7)$$

Copper (I) salts react with H_2O_2 to make ·OH radicals with a much greater rate constant than do Fe (II) salts:

$$Cu^+ + H_2O_2 \rightarrow Cu^{2+} + \cdot OH + OH^- \qquad (8)$$

Although free Mn^{2+} ions are present in some animal (Schramm, 1982) and bacterial (Archibald & Fridovich, 1981) cells, we have so far been unable to demonstrate an Mn^{2+}-dependent ·OH generation.

The hydroxyl radical reacts with extremely high rate constants with almost every type of molecule found in living cells: sugars, amino acids, phospholipids, DNA bases and organic acids. Its reactions are of three main types (Willson, 1978*a*). These are (i) hydrogen atom abstraction, e.g. for methanol:

$$CH_3OH + \cdot OH \rightarrow H_2O + \cdot CH_2OH \qquad (9)$$

(ii) addition, e.g. it can add on to aromatic ring structures such as the purine and pyrimidine bases of DNA, and (iii) electron transfer, e.g. with Cl^- ion:

$$Cl^- + \cdot OH \rightarrow Cl\cdot + OH^- \qquad (10)$$

The reactivity of ·OH radicals is so great that, if

they are formed in living systems, they will react immediately with whatever biological molecule is in their vicinity, producing secondary radicals of variable reactivity. For example, their reaction with carbonate ion (CO_3^{2-}) produces the carbonate radical ($CO_3^{-\cdot}$), a powerful reducing agent (Anbar & Neta, 1967).

If both H_2O_2 and Cu(I) and/or Fe(II) salts are available *in vivo*, then ·OH radicals will form. A biological system generating O_2^- will probably produce H_2O_2 (unless the O_2^- reacts immediately with another molecule) by the dismutation reaction, the rate of which will depend on the pH and on the concentration of O_2^-. H_2O_2 production, probably mainly via O_2^-, has been observed from whole bacteria of several species, from phagocytic cells, from spermatozoa (Holland *et al.*, 1982) and from mitochondria, microsomes and chloroplasts *in vitro* (Chance *et al.*, 1979; Halliwell, 1981*b*). Several enzymes, including glycollate and urate oxidases, produce H_2O_2 without the intermediacy of free O_2^- radical. Chance *et al.* (1979) calculated that 82nmol of H_2O_2 is produced/g of tissue per min in perfused livers isolated from normally fed rats. Inclusion of glycollate or urate in the perfusion medium increases this rate. The lens of the human eye contains micromolar concentrations of H_2O_2 (Bhuyan & Bhuyan, 1977) and H_2O_2 vapour has been detected in expired human breath (Williams & Chance, 1983), although it is possible that some or all of this might originate from oral bacteria (Thomas & Pera, 1983).

Hence H_2O_2 is available *in vivo*. What about metal ions?

Iron distribution: is iron available for the Fenton reaction?

An adult human contains about 4g of iron, about two-thirds of which is present in haemoglobin (for reviews see Harrison & Hoare, 1980; Aisen & Liskowsky, 1980). A further 10% is found in myoglobin and a very small amount in various iron-containing enzymes and the transport protein transferrin. The rest is present in intracellular storage proteins, ferritin and haemosiderin. These are found mainly in liver, spleen and bone-marrow, but to a limited extent in most other tissues and some ferritin is found in blood plasma.

Iron in the diet exists in the oxidized, Fe(III), form. The HCl in the stomach achieves solubilization and dietary vitamin C (ascorbic acid, a reducing agent) reduces some of the iron to the Fe(II) state and facilitates its absorption. Iron taken up by the gut enters the plasma protein transferrin, which functions as a carrier molecule. Transferrin is a glycoprotein and each molecule has two separate binding sites to which Fe(III) attaches extremely tightly. The binding is assisted by the presence at each site of an anion, usually HCO_3^- or CO_3^{2-}. Under normal conditions the transferrin present in the bloodstream is only about 30% loaded with iron on average, so that the amount of free iron salts available in the blood plasma would be expected to be virtually zero, a result confirmed by experiment (Gutteridge *et al.*, 1981*a*). A similar protein to transferrin, known as lactoferrin, is found in several body fluids and in milk and is produced by phagocytic cells (Reiter, 1979). Lactoferrin also binds two mol of Fe(III)/mol of protein.

Iron from transferrin must enter the various cells of the body for use in synthesizing iron enzymes and proteins. One view is that the protein is taken into iron-requiring cells by pinocytosis, so that it enters the cytoplasm in a vacuole. The contents of the vacuole are then acidified. This facilitates the release of iron, perhaps by decomposing the anion at the binding site. The iron released probably chelates to various cellular constituents such as citrate, ATP, GTP or other phosphate esters. The iron-free transferrin (apotransferrin) is ejected from the cell whilst the small pool of non-protein-bound iron can be used in the synthesis of iron proteins. For example, mitochondria take up iron salts rapidly for incorporation into cytochromes and non-haem-iron proteins and these organelles contain small 'pools' of chelated iron salts in the matrix (Tangeras *et al.*, 1980). On the other hand, some iron may leave transferrin immediately on attachment to the plasma membranes (Cole & Glass, 1983; Nunez *et al.*, 1983).

Any internal iron not required by cells is stored in ferritin. Ferritin has a molecular weight of 444000 and stores up to 4500mol of iron/mol of protein. It consists of a protein shell (apoferritin) enclosing the iron, in the form of a hydrated iron(III) oxide–phosphate complex. The protein shell has 24 subunits and six 'pores' that allow access to the interior. Ferritin appears to function in preventing an excessive intracellular accumulation of non-protein-bound iron, and synthesis of apoferritin can be stimulated by loading cells with iron salts. It is rarely saturated with iron *in vivo*. Iron enters ferritin as Fe(II), which becomes oxidized by the protein (perhaps with the aid of caeruloplasmin; see below) to Fe(III) and deposited in the interior. Similarly, iron can be removed from ferritin as Fe(II) by the action of a number of biological reducing agents, including cysteine, reduced flavins and ascorbate. Ferritin can be degraded in lysosomes, the protein shell being attacked to leave an insoluble product known as haemosiderin (Aisen & Liskowsky, 1980; Harrison & Hoare, 1980).

The small pool of non-protein-bound iron moving between transferrin, cell cytoplasm, mito-

chondria and ferritin could provide iron for the Fenton reaction. No doubt this is why the pool is kept as small as possible! In extracellular fluids, available iron at concentrations of μmol/l has been detected in human synovial fluid and in cerebrospinal fluid, but not in serum or plasma (Table 1). Available iron has been found in several bacteria (Gutteridge, 1984).

Thus both H_2O_2 and iron salts are available *in vivo* and, if they come into contact, the Fenton reaction will occur. Isolated liver microsomes, for example, contain sufficient iron and produce enough H_2O_2 to permit ·OH production (Cederbaum & Dicker, 1983).

Copper availability

The adult human body contains about 80mg of copper. It is absorbed from the diet in the stomach or upper small intestine, probably as complexes with amino acids, such as histidine, or small peptides (Pickart & Thaler, 1980). These copper complexes enter the blood and most of the copper binds tightly to serum albumin in equilibrium with a small 'pool' of copper complexes. In the liver the copper is taken up and incorporated into the glycoprotein caeruloplasmin, which is then released into the circulation (normal plasma content is 200–400mg/l). About 95% of total plasma copper is found in this protein, the rest being attached to albumin or amino acids as mentioned above (Harrison & Hoare, 1980). Caeruloplasmin contains 6 or 7mol of copper/mol of protein. It does not exchange copper readily nor will it tightly bind extra copper, so it does not have the characteristics of a copper transport protein [by contrast transferrin can easily bind Fe(III) and release it again within cells]. It seems that cells must take up and degrade caeruloplasmin in order to obtain copper from it (Gutteridge & Stocks, 1981).

Caeruloplasmin can catalyse the oxidation of a variety of polyamines and polyphenols, the oxygen taken up being reduced to water. It will also oxidize Fe(II) ions to Fe(III) ions (its ferroxidase activity) and again water is produced. The biological significance of these enzyme activities remains uncertain. Suggestions that the ferroxidase activity of caeruloplasmin is of importance in allowing iron to bind to transferrin have not been unanimously accepted (Gutteridge & Stocks, 1981). Another suggestion (Boyer & Schori, 1983) is that it helps load iron into ferritin.

So is free copper ion available to make ·OH from H_2O_2? Addition of Cu(I) salts to a system generating H_2O_2 *in vitro* causes formation of free ·OH radicals, but this is prevented by added histidine or albumin at physiological concentrations (Rowley & Halliwell, 1983*a*). Hence the copper ions in blood serum cannot give rise to 'free' ·OH radicals. Similarly, the protein concentration within the cells is very high and if any free copper ions are available they should rapidly become protein-bound and unable to catalyse formation of free ·OH radicals. By contrast, albumin does not prevent Fe(II)-dependent formation of free ·OH radicals (Halliwell, 1978*a*). It may be, however, that the bound copper ions can still generate ·OH which reacts immediately with the binding molecule and thus cannot be detected in free solution as suggested by Samuni *et al.* (1981). Consistent with this, mixtures of copper salts and H_2O_2 will damage many proteins (Gutteridge & Wilkins, 1983).

The superoxide theory of oxygen toxicity; is the superoxide radical a toxic species?

The superoxide theory of O_2 toxicity has been given an enormous boost by the accumulation of

Table 1. *'Bleomycin-detectable' iron in extracellular fluids*
The presence of non-protein-bound iron is tested for by utilizing the fact that the antibiotic bleomycin requires the presence of iron salts in order to degrade DNA. Results are taken from Gutteridge *et al.* (1981*a*, 1982). This assay does not detect iron bound to proteins such as transferrin or ferritin. Indeed, addition of native transferrin makes the iron in body fluids unavailable for the bleomycin reaction (see below). Bleomycin-detectable iron presumably exists *in vivo* bound to low-molecular-weight chelating agents such as citrate and ATP. The existence of low-molecular-weight iron complexes detectable by bleomycin suggests that the normal iron-binding proteins in body fluids are either fully saturated (as may be the case in cerebrospinal fluid) or are defective in their ability to bind iron (as may be the case in rheumatoid arthritis).

Fluid tested	Concentration (μmol/l) of non-protein-bound iron salts (mean ± S.D.)
Human blood serum or plasma	0
Human synovial fluid* (rheumatoid patients)	2.8 ± 1.2
Synovial fluid + 10 μM-apotransferrin	0
Human cerebrospinal fluid (normal)	2.2 ± 1.3
Cerebrospinal fluid + 10 μM-apotransferrin	0
Inflammatory exudate fluid (caused by inducing inflammation in rats)	0.5, 1.2 (two typical samples)

* The iron content of normal synovial fluid has not been established since there is no ethical reason for taking samples from normal joints. Some iron is present in synovial fluid from patients with osteoarthritis.

evidence showing that superoxide dismutase (SOD) enzymes, which remove O_2^- by accelerating the dismutation reaction, are of great importance in allowing organisms to survive in the presence of O_2 and to tolerate increased O_2 concentrations (for reviews see Fridovich, 1975, 1978, 1983; Halliwell, 1981*a*,*b*, 1983). Since SOD enzymes are specific for O_2^- as substrate, it follows that O_2^- must be a toxic species. Indeed, O_2^--generating systems have many damaging effects, several of which are summarized in Table 2. Why should this be so?

Superoxide chemistry differs greatly according to whether reactions are carried out in aqueous solution or in organic solvents, in many of which it is very stable. In nonpolar environments O_2^- is a powerful base (proton acceptor), nucleophile, and reducing agent (Frimer, 1982; Sawyer & Valentine, 1981). For example, it will reduce dissolved SO_2 gas:

$$SO_2 + O_2^- \rightarrow O_2 + SO_2^- \qquad (11)$$

and it will even displace Cl^- ion from such unreactive chlorinated hydrocarbons as CCl_4 (Roberts & Sawyer, 1981). Superoxide can also act as an oxidizing agent, but this ability is only seen with compounds that can donate H^+ ions such as catechol, ascorbate or α-tocopherol (vitamin E).

Table 2. *Some deleterious effects of systems generating the superoxide radical*
Reviews of the papers from which the data are taken can be mostly found in Halliwell (1981*b*) and Michelson *et al.* (1977).

Source of O_2^-	System studied	Damage	Comments
Heart-muscle submitochondrial particles	Activity of NADH–CoQ reductase complex	Activity lost	Damage prevented by SOD. The O_2^- generated by the complex inactivates it unless SOD is present. Catalase not protective
Illuminated FMN	Bacteria	Loss of viability	Protection by SOD
Xanthine + xanthine oxidase	Human synovial fluid	Degradation; loss of viscosity	Both SOD and catalase protect
Xanthine + xanthine oxidase	Bacteriophage R17	Inactivation	SOD protects partially
Illuminated FMN	Ribonuclease	Loss of activity	SOD protects partially
Illuminated FMN	Calf myoblast cells	Growth abnormality, some cell death	SOD protects partially
Hypoxanthine + xanthine oxidase	Rat brain membrane (Na^+,K^+)-ATPase	Inactivation	SOD protects partially
Acetaldehyde + xanthine oxidase	Erythrocyte membranes	Lysis	SOD protects
Acetaldehyde + xanthine oxidase	Arachidonic acid	Oxidation	Both SOD and catalase protect
Hypoxanthine + xanthine oxidase	DNA	Degradation, single-strand breaks, attack on sugar moiety	SOD, ·OH scavengers and catalase protect. Iron salts needed
Autoxidation of dihydroxyfumarate	Rat thymocytes	Inhibition of Na^+-dependent amino acid uptake	SOD protects, but not catalase
Hypoxanthine + xanthine oxidase	Cheek pouch of living hamster (perfused with O_2^--generating system)	Increased permeability of blood vessels, leakage of contents	SOD protects
Autoxidation of dialuric acid	*Escherichia coli*	Loss of viability	Both SOD and catalase protect
Acetaldehyde + xanthine oxidase	*Staphylococcus aureus*	Loss of viability	SOD, ·OH scavengers, catalase protect. Traces of iron chelate needed for killing
Xanthine + xanthine oxidase	Rat lung *in vivo* (instilled into lungs)	Acute lung injury, oedema	SOD protects but not catalase
Xanthine + xanthine oxidase	Rat heart ornithine decarboxylase	Inactivation	SOD protects, also mannitol (·OH scavenger)
Xanthine + xanthine oxidase	Rat heart mitochondria	Lowered P/O ratios and lower respiratory control	SOD and catalase protect
Xanthine + xanthine oxidase	Rat heart or liver mitochondria	Inhibition of net Ca^{2+} uptake	Protected SOD or mannitol (·OH scavenger)
Xanthine + xanthine oxidase	Dog heart sarcoplasmic reticulum	Decreased Ca^{2+} uptake	Some protection by SOD and mannitol

The O_2^- becomes protonated and the substrate radical is then oxidized by the resulting hydroperoxyl radical ($HO_2^\cdot$) or by molecular O_2. For example, the mechanism of α-tocopherol (Ht) oxidation can be written (Nanni *et al.*, 1980):

$$O_2^- + Ht \rightarrow t^- + HO_2^\cdot \quad (12)$$

$$O_2^- + HO_2^\cdot \rightarrow HO_2^- + O_2 \quad (13)$$

$$O_2 + t^- \rightarrow O_2^- + t^\cdot \quad (14)$$

$$2t^\cdot \rightarrow \text{dimer} \rightarrow \text{other products} \quad (15)$$

In aqueous solution the basic properties and nucleophilicity of O_2^- are greatly reduced, as is its oxidizing capacity. Its major property is then to act as a reducing agent. For example, it reduces Fe(III) ions at the active site of cytochrome *c* and Cu(II) ions at the active site of plastocyanin. Any reaction undergone by O_2^- in aqueous solution will be in competition with the dismutation reaction.

The interior of biological membranes is well-known to be hydrophobic, and O_2^- produced in this environment could be extremely damaging, e.g. destroying phospholipids by a nucleophilic attack upon the carbonyl groups of the ester bonds linking fatty acids to glycerol (Niehaus, 1978). Much of the O_2^- generated within cells comes from membrane-bound systems (Halliwell, 1981*b*) and it is certainly possible that some of it is formed in the membrane interior, especially as O_2 is much more soluble in organic solvents than it is in water.

However, the effects listed in Table 2 were produced by O_2^- in aqueous solution. In view of its poor reactivity, it seems unlikely that O_2^- alone can do the damage, and in many cases protection is seen not only with SOD but also with catalase (Table 2). It was therefore suggested (Fridovich, 1975) that O_2^- and H_2O_2 interact to form the highly-reactive hydroxyl radical according to the overall equation:

$$H_2O_2 + O_2^- \rightarrow O_2 + OH^- + \cdot OH \quad (16)$$

Indeed, scavengers of ·OH such as mannitol, formate and thiourea can often protect against damage induced by O_2^--generating systems (Table 2). Formation of ·OH in a wide range of systems generating O_2^- has been detected by the ability of this radical to hydroxylate aromatic compounds (Halliwell, 1978*a*; Richmond *et al.*, 1981), attack tryptophan (McCord & Day, 1978; Singh *et al.*, 1981), give a characteristic e.s.r. signal with spin traps (Finkelstein *et al.*, 1979), convert methional into ethene (Beauchamp & Fridovich, 1970), dimethylsulphoxide into formaldehyde and methane (Klein *et al.*, 1981) and deoxyribose into thiobarbituric-acid-reactive material (Halliwell & Gutteridge, 1981) and to decarboxylate benzoic acid (Sagone *et al.*, 1981).

Reaction (16) was first postulated by Haber & Weiss (1934) and has become known as the Haber–Weiss reaction. Unfortunately the rate constant for the reaction in aqueous solution has since been shown to be virtually zero (for a review see Halliwell, 1981*b*) and it certainly could not occur at the low steady-state concentrations of O_2^- and H_2O_2 present *in vivo*. Several scientists (see references above) have shown that ·OH formation can be accounted for if the Haber–Weiss reaction is catalysed by traces of transition metal ions. Since iron salts are available *in vivo* (see above and Table 1) a plausible mechanism can be written as follows:

$$Fe^{3+} + O_2^- \rightarrow Fe^{2+} + O_2$$
(O_2^- reducing the iron salt) (17)

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^-$$
(Fenton reaction) (18)

$$\text{Net: } O_2^- + H_2O_2 \xrightarrow[\text{catalyst}]{\text{Fe salt}} O_2 + \cdot OH + OH^- \quad (19)$$

Reaction (19) is sometimes called an iron-catalysed Haber–Weiss reaction. Gutteridge *et al.* (1979) showed that the iron chelator desferrioxamine binds Fe(III) tightly so that it cannot be reduced by O_2^-, and hence it inhibits ·OH formation. Desferrioxamine is virtually specific for the chelation of Fe(III) ions [it can bind Al^{3+} ions and indeed it has been used to decrease Al^{3+} toxicity in patients undergoing blood dialysis (Brown *et al.*, 1982), but Al^{3+} does not catalyse ·OH formation]. Desferrioxamine can be injected subcutaneously into humans in amounts up to 50–60 mg/kg (Davies *et al.*, 1983) and it is a valuable experimental tool for investigation of the iron-catalysed Haber–Weiss reaction. Care must be taken in such experiments to keep the concentration at 1 mM or below since desferrioxamine, like most other compounds, reacts directly with ·OH (Hoe *et al.*, 1982; Willson, 1982).

The less-specific chelating agent bathophenanthroline sulphonate (Halliwell, 1978*b*) also inhibits the iron-catalysed Haber–Weiss reaction. By contrast, EDTA promotes the reaction (McCord & Day, 1978; Halliwell, 1978*a*) since Fe(III)–EDTA chelates are reduced by O_2^- quite quickly (Butler & Halliwell, 1982). Rosen & Klebanoff (1981) pointed out that many scientists studying ·OH formation had added EDTA to their systems either deliberately (e.g. McCord & Day, 1978) or accidentally by using commercial reagents that contained EDTA, such as some xanthine oxidase preparations. However, ·OH formation by the iron-catalysed Haber–Weiss reaction still occurs in systems containing no EDTA (Halliwell & Gutteridge, 1981; Richmond, 1983). Flitter *et al.* (1983) investigated the effect of EDTA and of the physiological chelator ATP on ·OH formation at different iron concentrations. They found that at physiological (μM) iron salt concentrations, ·OH will be

formed in the absence of EDTA. Floyd (1983) has also reported the ability of ATP or ADP iron chelates to form ·OH radicals. Complexes of iron (III) with picolinic acid, a metabolite of tryptophan, are catalysts of ·OH formation (Bannister *et al.*, 1983).

The chelating agent DETAPAC was reported by Buettner *et al.* (1978) to inhibit the iron-catalysed Haber–Weiss reaction. Fe(III)–DETAPAC complexes are reduced by O_2^- more slowly than Fe(III)–EDTA complexes (Butler & Halliwell, 1982). However, Richmond (1983) has pointed out that most of the test systems used contained EDTA and has claimed that if EDTA is removed then the inhibitory effect of DETAPAC is less marked. DETAPAC can either stimulate or inhibit iron-dependent lipid peroxidation, depending on its concentration (Gutteridge *et al.*, 1979). In view of these ambiguities and of the observation that DETAPAC chelates several other metal ions, we must point out that the widespread use of DETAPAC, e.g. in spin-trapping experiments, to suppress iron-dependent ·OH formation might give misleading results. Desferrioxamine (1 mM or less) should be used instead (Gutteridge *et al.*, 1979; Hoe *et al.*, 1982).

No iron salts are present in normal blood serum or plasma, but will iron-proteins catalyse ·OH formation? Native haemoglobin, methaemoglobin cytochromes, horseradish peroxidase or myeloperoxidase will not (Halliwell, 1978a). Ambruso & Johnston (1981) reported that iron-saturated lactoferrin is a catalyst, an observation confirmed by Bannister *et al.* (1982*a*), although Winterbourn (1983) has pointed to some artefacts in the assay systems used and has concluded that it is at best a weak catalyst. Ward *et al.* (1983) found that apolactoferrin or desferrioxamine could decrease complement- and neutrophil-mediated lung injury in rats. Iron(III) salts potentiated the damage, but iron-loaded lactoferrin did not. Iron-saturated lactoferrin contains 2 mol of Fe(III)/mol of protein, but *in vivo* the average iron loading of the protein is 20% or less, i.e. there will be only a small proportion of molecules with 2 mol of Fe(III) actually bound. If only 1 mol of Fe(III) is bound, lactoferrin is not a catalyst (Winterbourn, 1983; Gutteridge *et al.*, 1981*b*). Iron-loaded transferrin has been reported to catalyse ·OH formation (McCord & Day, 1978; Bannister *et al.*, 1982*b*; Motohashi & Mori, 1983) but partially-iron loaded protein does not (Maguire *et al.*, 1982). The same may be true of ferritin, perhaps because O_2^- acting as a reducing agent can mobilize iron from the iron-loaded protein (Bannister *et al.*, 1984). Some denaturation products of haemoglobin appear to promote ·OH formation (Hebbel *et al.*, 1982).

Hydroxyl radicals, once generated, will react with the molecules in their immediate surroundings. Superoxide and H_2O_2 are less reactive and can diffuse away from their sites of formation, leading to ·OH generation in different parts of the cell whenever they meet a 'spare' transition metal ion. In a sense, therefore, they can be damaging because of their poor reactivity. H_2O_2 also crosses all cell membranes easily, whereas O_2^- does not unless it can pass through a specific 'channel'. Hydroxyl radical will never pass through: it reacts with the first membrane component it meets.

Ferryl and perferryl radicals

Identification of the reactive radical produced in the Fenton reaction as ·OH was at first disputed, although it is now generally accepted. For example, formation of a ferryl radical in which the iron has an oxidation number of 4 was suggested to occur instead (Walling, 1982):

$$Fe^{2+} + H_2O_2 \rightarrow FeOH^{3+} \text{ (or } FeO^{2+}) + OH^- \quad (20)$$

Ferryl radical may be the reactive species at the active sites of horseradish peroxidase compounds I and II (Dunford, 1982) and of cytochrome *P*-450 (Sligar *et al.*, 1982) and it must therefore have considerable reactivity. Hence it is not surprising that this controversy has spilled over into discussion of the iron-catalysed Haber–Weiss reaction, in which ·OH is produced by the Fenton reaction. However, if the reactive species produced in O_2^--generating systems is not ·OH, then it must still attack spin traps to produce the correct ·OH signal, react with scavengers of ·OH with similar rate constants to ·OH itself (Anbar & Neta, 1967) and require both O_2^- and H_2O_2 for its formation. Ferryl radicals have not yet been demonstrated to do any of these things. This is not to say, however, that ·OH is the only oxidizing radical found in systems containing O_2^-, H_2O_2 and iron salts. This point is illustrated by studies of lipid peroxidation (see below).

Another species often mentioned is perferryl radical, FeO^+ or Fe^{3+}–O_2^-. It is found at the active site of peroxidase compound III, but its oxidizing capacity is much lower than that of ferryl or ·OH radicals (Tamura & Yamazaki, 1972). Perferryl is a minor contributor to the resonance structure that results when O_2 binds to Fe(II) in myoglobin and haemoglobin (Fe^{2+}–$O_2 \longleftrightarrow Fe^{3+}$–$O_2^-$).

Criticisms of the metal-catalysed Haber–Weiss reaction as an explanation of O_2^- toxicity

In the iron-catalysed Haber–Weiss reaction, the only role apparent for O_2^- is to reduce Fe(III) to Fe(II), which is consistent with its chemistry in aqueous solution. Several scientists (e.g. Fee, 1982; Winterbourn, 1979, 1981) have argued that this

could not happen *in vivo* because the concentration of other biological reducing agents would be greater than that of O_2^-. Rowley & Halliwell (1982*a*,*b*) investigated this possibility and concluded that NADPH, NADH or thiol compounds such as GSH and cysteine would not prevent formation of ·OH *in vivo*. Indeed, both thiol compounds (Rowley & Halliwell, 1982*a*; Searle & Tomasi, 1982) and NAD(P)H (Rowley & Halliwell, 1982*b*) can interact with metal ions and H_2O_2 to increase ·OH formation under certain circumstances.

The situation with ascorbic acid is more complicated (Winterbourn, 1979; Rowley & Halliwell, 1983*b*). Ascorbate at the concentrations normally present in extracellular fluid can at best partially replace O_2^- in reducing Fe(III), but it is rapidly oxidized by direct reaction with O_2^- (Nishikimi, 1975) and with ·OH (Anbar & Neta, 1967) so that ·OH production eventually becomes completely O_2^--dependent (Rowley & Halliwell, 1983*b*). By contrast, at the very high ascorbate concentrations present in a few body tissues such as the eye (Varma *et al.*, 1977) or pneumocytes (Castranova *et al.*, 1983) it is difficult to envisage O_2^- competing as a reductant unless the ascorbate and O_2^- are present in different subcellular compartments or O_2^- is produced in very large quantities. The latter situation can certainly occur, e.g. in the 'shock lung' syndrome large numbers of neutrophils accumulate in the lungs, aggregate and become activated to produce O_2^- (Repine *et al.*, 1982).

Mixtures of copper salts and H_2O_2 in the presence of a reducing agent also produce free ·OH radicals. The binding of Cu(II) to physiological ligands such as histidine or albumin does not prevent its reduction by O_2^- (Brigelius *et al.*, 1974) and so it is likely that ·OH radicals are still formed and immediately attack the binding molecule (Samuni *et al.*, 1984).

Inspection of Table 2 shows that the metal-dependent Haber–Weiss reactions do not explain everything, as there are several damaging effects prevented by SOD but not by catalase. A further illustration of this comes from the organism *Streptococcus sanguis*, whose growth appears to be independent of the availability of iron (Diguiseppi & Fridovich, 1982). It contains no haem compounds and lacks catalase and peroxidase. It is damaged by exposure to a O_2^--generating system but damage is not prevented by scavengers of ·OH.

Dismutation of O_2^- catalysed by the SOD enzymes produces H_2O_2 and O_2 in the ground state. An additional explanation that has been offered to explain the toxicity of O_2^--generating systems is that they produce singlet O_2 $^1\Delta g$, either in the non-enzymic dismutation reaction of O_2^- or in the metal catalysed Haber–Weiss reaction (which would account for inhibition by both SOD and catalase) (Kellogg & Fridovich, 1977). If singlet O_2 is produced in either of these reactions, however, the amount is very small and difficult to detect. Indeed, O_2^- itself is a quencher of singlet O_2 by electron transfer (Khan, 1977, 1981):

$$O_2^- + O_2^* \rightarrow O_2 + O_2^- \quad (21)$$

Finally, it should be noted that the protonated form of O_2^-, $HO_2^\cdot$, is a more reactive radical than is O_2^- in aqueous solution (Gebicki & Bielski, 1981). The pK_a of $HO_2^\cdot$ in aqueous solution is about 4.8 and so the amount present at physiological pH will be small, although perhaps enough to exert some biological effects. In nonpolar environments such as the interior of membranes, the formation of $HO_2^\cdot$ will be more favoured. It might also be formed in the phagocytic vacuole of neutrophils, which operates at an acid pH and the microenvironment at the surface of activated macrophages can have pH < 5 (Etherington *et al.*, 1981).

In conclusion, therefore, O_2^--generating systems are unpleasant for a number of reasons, principally the formation of more-reactive species such as ·OH. The O_2^- radical itself, however, can interact with extracellular fluids to generate a factor chemotactic for neutrophils (McCord *et al.*, 1982), an effect that would potentiate inflammation.

Oxygen radicals, metal ions and lipid peroxidation

Initiation of lipid peroxidation in a membrane or free fatty acid is due to the attack of any species that has sufficient reactivity to abstract a hydrogen atom (e.g. eqn. 22). Since a hydrogen atom has only one electron this leaves behind an unpaired electron on the carbon atom. The carbon radical in a polyunsaturated fatty acid tends to be stabilized by a molecular rearrangement to produce a conjugated diene, which rapidly reacts with O_2 to give a hydroperoxy radical (eqn. 23). Hydroperoxy radicals abstract hydrogen atoms from other lipid molecules and so continue the chain reaction of lipid peroxidation (eqn. 24). The hydroperoxy radical combines with the hydrogen atom that it abstracts to give a lipid hydroperoxide (eqn. 24):

$$\text{Lipid-H} + \cdot\text{OH} \rightarrow \text{Lipid}^\cdot + H_2O \quad (22)$$

$$\text{Lipid}^\cdot + O_2 \rightarrow \text{Lipid-}O_2^\cdot \quad (23)$$

(after molecular rearrangement)

$$\text{Lipid-}O_2^\cdot + \text{Lipid-H} \rightarrow \text{Lipid-}O_2\text{H} + \text{Lipid}^\cdot \quad (24)$$

Detailed reviews of lipid peroxidation may be found in Barber & Bernheim (1967) and McBrien & Slater (1982) so we shall consider here only the part played by metals and oxygen radicals in the peroxidation process.

Decomposition of lipid hydroperoxides: the propagation of lipid peroxidation

Pure lipid hydroperoxides are fairly stable at physiological temperatures and a major role of transition metals is to catalyse their decomposition. Many metal complexes that can do this are present *in vivo*. They include simple complexes of iron salts with phosphate ion or phosphate esters such as ADP. Also effective are haem, haemoproteins such as haemoglobin, methaemoglobin, peroxidase, cytochrome *P*-450, other cytochromes and non-haem iron proteins (O'Brien, 1969; Kaschnitz & Hatefi, 1975; Gutteridge, 1977; Aust & Svingen, 1982). All these should contribute to the propagation of lipid peroxidation in membranes *in vivo*. Lactoferrin and transferrin do not stimulate hydroperoxide decomposition unless they are fully iron-loaded and then the effect is weak at best (Gutteridge *et al.*, 1981*b*), whereas ferritin stimulates to an extent increasing with its iron loading (Gutteridge *et al.*, 1983). It is thus advantageous for cells to keep iron salts bound to these proteins in such a way that their iron-loading never approaches a maximum, a strategy that seems to be used in the human body (see above).

Reduced iron compounds react with lipid hydroperoxides (lipid-O_2H) to give alkoxy (lipid-$O^{\cdot}$) radicals.

$$\text{Lipid-}O_2H + Fe^{2+}\text{-complex} \rightarrow Fe^{3+}\text{-complex} + OH^- + \text{lipid-}O^{\cdot} \quad (25)$$

With an iron(III) compound a peroxy (lipid-$O_2^{\cdot}$) radical will form:

$$\text{Lipid-}O_2H + Fe^{3+}\text{-complex} \rightarrow \text{lipid-}O_2^{\cdot} + H^+ + Fe^{2+}\text{-complex} \quad (26)$$

Both alkoxy and peroxy radicals stimulate the chain reaction of lipid peroxidation by abstracting further hydrogen atoms (eqn. 24). EDTA or DETAPAC either increase or inhibit lipid peroxidation stimulated by iron salts depending on the ratio of the concentration of chelator to that of iron salt, whereas desferrioxamine inhibits at all concentrations tested (Wills, 1969; Gutteridge *et al.*, 1979). Copper salts also stimulate peroxide decomposition (Gutteridge, 1977; Sree Kumar *et al.*, 1978).

Initiation of lipid peroxidation

Scientists often refer to metal salts and their complexes as 'initiating' peroxidation, but in most cases what is happening is that they are causing the decomposition of pre-formed lipid hydroperoxides. All commercially available unsaturated fatty acids contain lipid hydroperoxides. Often it is not clear how these have arisen, but one mechanism for forming them is a direct reaction of singlet $O_2{}^1\Delta g$ with membrane lipids (Rawls & Van Santen, 1970). Illumination of unsaturated fatty acids in the presence of sensitizers of singlet O_2 formation such as chlorophyll, porphyrins, bilirubin or retinal initiates rapid peroxidation and such reactions occur *in vivo* in the mammalian eye, the illuminated chloroplast and in patients suffering from porphyrias (for reviews see Foote, 1982; Krinsky & Deneke, 1982).

It has been further suggested that singlet O_2 is formed during the complex degradation reactions of lipid peroxidation and might contribute to the chain reaction by causing more initiation. The evidence for this singlet O_2 formation is unconvincing, however, since it was obtained by using 'singlet O_2 scavengers' that are by no means specific for reaction with this species (for a review see Halliwell, 1981*b*). Indeed, a number of 'singlet O_2 scavengers' react with at least one organic peroxy radical (Packer *et al.*, 1981). Nevertheless, something with an oxidizing capacity comparable to that of singlet O_2 $^1\Delta g$ is formed during lipid peroxidation in some systems and it is probably best to call it a 'singlet O_2-like factor' (Lai *et al.*, 1978).

A number of scientists have observed that O_2^--generating systems stimulate the peroxidation of fatty acids or of membranes. Fridovich & Porter (1981) found that a mixture of xanthine oxidase and its substrate ethanal oxidizes arachidonic acid. This was inhibited by mannitol (a ·OH scavenger), superoxide dismutase, catalase and DETAPAC, which suggests that peroxidation was stimulated by ·OH radicals formed by an iron-catalysed Haber–Weiss reaction. Hydroxyl radicals are known to be capable (eqn. 22) of abstracting hydrogen atoms from membrane lipids (Fong *et al.*, 1973). Barber & Thomas (1978) measured a rate constant of $5 \times 10^8\,M^{-1}\cdot s^{-1}$ for the reaction of ·OH with artificial lecithin bilayers. Superoxide itself is insufficiently reactive in aqueous solution to abstract hydrogen atoms from fatty acids (see above). It is possible, however, that $HO_2^{\cdot}$ can attack fatty acids directly and evidence for conversion of linoleic, linolenic and arachidonic acids to their hydroperoxides by $HO_2^{\cdot}$ has been presented by Bielski *et al.* (1983). Another suggestion (Thomas *et al.*, 1982) is that O_2^-, or perhaps $HO_2^{\cdot}$, decompose lipid hydroperoxides:

$$\text{Lipid-}O_2H + O_2^- \rightarrow \text{lipid-}O^{\cdot} + OH^- + O_2 \quad (27)$$

$$\text{Lipid-}O_2H + HO_2^{\cdot} \rightarrow \text{lipid } O^{\cdot} + H_2O + O_2 \quad (28)$$

Iron(II) and its complexes stimulate peroxidation more than does iron(III), probably in part because alkoxy radicals are more reactive than peroxy radicals in initiating peroxidation. A second reason is that reduced iron chelates in the presence of air generate oxygen radicals, including O_2^- and ·OH (Michelson *et al.*, 1977; Halliwell, 1978*b*; Halliwell & Gutteridge, 1981):

$$Fe^{2+}(aq.)+O_2 \leftrightarrows Fe^{2+}-O_2 \leftrightarrow Fe^{3+}-O_2^- \quad \text{(perferryl)}$$
$$\rightleftarrows Fe^{3+}+O_2^- \quad (29)$$
$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \quad \text{(dismutation)} \quad (30)$$
$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + \cdot OH + OH^- \quad \text{(Fenton reaction)} \quad (31)$$

The rate of peroxidation of purified membrane lipids or microsomal fractions in the presence of Fe(III) chelates can be accelerated by adding ascorbic acid, a reducing agent (Wills, 1966) and sometimes by adding thiol compounds (Tien *et al.*, 1982; Searle & Willson, 1983). The NADPH–cytochrome *P*-450 reductase enzyme of microsomal fractions reduces a number of Fe(III) chelates, such as Fe(III)–ADP (Aust & Svingen, 1982) to achieve the same effect. In all these systems ·OH radicals are formed in the reaction mixture (Lai *et al.*, 1979; Gutteridge, 1982; Rowley & Halliwell, 1982*a*,*b*; Searle & Tomasi, 1982) but addition of scavengers of these radicals has little effect on the observed rate of peroxidation (Aust & Svingen, 1982; Gutteridge, 1982). The most sensible explanation of all these results seems to be that although ·OH radicals are being generated in the reaction mixtures, they are not contributing much to the initiation of peroxidation. Aust & Svingen (1982) have suggested that perferryl can initiate, but in view of its poor reactivity a ferryl radical seems more likely. If ferryl is the true initiator, then the lack of effect of ·OH scavengers on peroxidation means that reactions due to the ferryl radical can be easily distinguished from those due to ·OH in biochemical systems.

Why the ·OH radicals known to be formed in the above systems do not appear to initiate peroxidation although they can in other systems is not clear. In microsomes they might preferentially attack other membrane components (e.g. proteins) but this could not explain the results with purified membrane lipids (Gutteridge, 1982). Perhaps they react with the hydrophilic 'head groups' of the lipids and rarely penetrate to reach the fatty acid side-chains.

Transition metals, oxygen radicals and some diseases

Iron overload

The amount of iron in the human body is determined by the amount assimilated from the gut; there is no obvious physiological mechanism for disposing of excess iron. The consequences of this are seen in iron overload. This can happen as a result of grossly excessive dietary iron intake, as in the Bantu tribe of Africa who drink acidic beer out of iron pots (Nienhuis, 1981) or as a result of an inherited disease (idiopathic haemochromatosis) in which gut absorbtion of iron is abnormally high. It can also be caused by medical treatments of other diseases. For example, the thalassaemias are inborn conditions in which the rate of synthesis of one of the haemoglobin chains is diminished, the prefix α- or β-thalassaemia being used to identify the chain that is synthesized abnormally slowly (Jacobs, 1977).

Untreated patients with thalassaemia major (the homozygous state) die of anaemia in infancy, but can be kept alive by regular blood transfusions. Since each unit of blood contains about 0.2g of iron, the patients become overloaded with iron, leading to saturation of ferritin and transferrin and often the appearance of non-protein-bound iron complexes in the blood (Hershko *et al.*, 1978). Iron accumulates especially in the liver, spleen and heart, the latter organ being very sensitive to it so that many thalassaemics treated by transfusion suffer cardiac malfunctions (Schafer *et al.*, 1981). Similar problems arise in the treatment of other chronic anaemias by transfusion. Studies of the pathology of iron overload point strongly to the importance of free radical reactions, of which lipid peroxidation and the formation of ·OH from iron salts and H_2O_2 are of major importance (for reviews see Young & Aisen, 1982; Bacon *et al.*, 1983). The authors found a non-protein-bound iron concentration of 26 μM in the serum of one patient with idiopathic haemochromatosis. Treatment of iron overload often involves use of desferrioxamine. Children with β-thalassaemia major in Britain were first given desferrioxamine in 1962, and it appears to have been successful in prolonging their lifespan (Modell *et al.*, 1982). Desferrioxamine and its iron(III) complex (ferrioxamine) are rapidly excreted, mainly in urine but also in bile, so removing iron from the body. Because desferrioxamine cannot be given by mouth and is only slowly taken up by cells there is now interest in other chelators such as rhodotorulic acid and pyridoxal isonicotinoyl hydrazone (Jones, 1982).

Patients with iron overload, and healthy Bantu, often have abnormally low contents of ascorbic acid in blood and tissues. Feeding them with ascorbate in the absence of desferrioxamine has produced deleterious and sometimes lethal consequences, possibly because of increased lipid peroxidation and generation of ·OH by ascorbate/iron salt mixtures (Nienhuis, 1981). Simultaneous administration of desferrioxamine should prevent these effects.

Rheumatoid arthritis

The production of oxygen radicals during the respiratory burst of phagocytic cells plays an essential role in bacterial killing and in regulating the process of acute inflammation (Babior, 1978*a*,*b*; Halliwell, 1982) by changes in vascular perme-

ability (Del Maestro *et al.*, 1982) and the generation of chemotactic factors (McCord *et al.*, 1982). In view of the damaging effects of oxygen radicals on tissues (Table 2), it follows that anything causing abnormal activation of phagocytes has the potential to provoke a devastating response. The most striking consequences of this are seen in the autoimmune diseases (for reviews see Halliwell, 1982; Theofilopoulos & Dixon, 1982) in which antibodies against normal body constituents are formed and provoke attack by phagocytic cells.

Rheumatoid arthritis has many features of an autoimmune disease although its exact cause is unknown. The blood serum and joint fluid often contains an antibody (rheumatoid factor) that binds to immunoglobulin G. The onset of rheumatoid arthritis is usually slow. The synovium of the joints becomes swollen and damaged and joint cartilage is eroded. Production of synovial fluid, the natural joint lubricant, is increased but its viscosity is much below normal and its lubricating capacity is greatly diminished. The decrease in viscosity is due to breakdown of the polymer hyaluronic acid. Exposure of synovial fluid to O_2^- produced by chemical systems or by activated phagocytes *in vitro* produces a similar breakdown, which can be attributed to the O_2^--dependent formation of ·OH by an iron-catalysed Haber–Weiss reaction in the synovial fluid (McCord, 1974; Halliwell, 1978*b*, Greenwald & Moy, 1980). The synovial fluid of rheumatoid joints swarms with neutrophils and contains micromolar quantities of iron salts (Table 1), but has little superoxide dismutase or catalase activities (McCord, 1974; Blake *et al.*, 1981*a*). Lactoferrin, presumably released from neutrophils, is also present in increased amounts, as are products of lipid peroxidation (Lunec & Dormandy, 1979).

That rheumatoid arthritis is accompanied by abnormalities in body iron metabolism has been known for many years (e.g. Muirden, 1970). A rapid fall in the 'total iron' content of blood serum at the onset of inflammation is followed by a drop in haemoglobin concentration and increased deposition of iron proteins in the synovial membranes (Blake *et al.*, 1981*b*). The drop in serum iron correlates closely with the activity of the inflammatory process. The iron in the synovial membrane is largely present within ferritin. In early rheumatoid disease the presence of ferritin and haemosiderin iron implies a poor prognosis (Blake *et al.*, 1981*b*). Attempts to reverse the 'anaemia of rheumatoid arthritis' by giving oral iron salts to patients are usually ineffective in the absence of improvement in their disease, and such oral treatment has been shown to worsen the symptoms of at least one patient (Blake & Bacon, 1982). Intravenous iron therapy frequently causes problems.

Loss of 'total iron' from the blood has been observed in many other inflammatory conditions, in other forms of tissue injury and during infections. It may represent an attempt by the body to withhold iron from invading bacteria or what it thinks are invading bacteria (Weinberg, 1978; Bullen, 1981). However, in view of the importance of non-protein-bound iron salts, and possibly lactoferrin, in promoting ·OH formation and of the ability of iron bound to ferritin to stimulate lipid peroxidation, it seems likely that this redistribution of body iron potentiates the inflammatory process. Injection of large doses of desferrioxamine or other iron chelators into animals suffering induced inflammation often produces a worsening of acute inflammation but a suppression of chronic inflammation (e.g. Blake *et al.*, 1983). Whether or not these effects of chelators are related to oxygen radical reactions remains to be established, but they may be of therapeutic interest.

Cancer

Any substance that reacts with DNA is potentially carcinogenic. Exposure of DNA to O_2^--generating systems causes extensive strand breakage and degradation of deoxyribose (e.g. Brawn & Fridovich, 1981), probably due to formation of ·OH. Oxygen radicals have been suggested to be involved in the action of a number of DNA-damaging drugs (Lown *et al.*, 1982). Indeed, exposure of mammalian cells to activated human neutrophils produces chromosome damage (Weitberg *et al.*, 1983) and the DNA within neutrophils themselves is fragmented during phagocytosis (Birnboim, 1982). The mutagenic effects of O_2 radicals produced during the respiratory burst may promote the development of cancer in chronically-inflamed tissues.

The role of transition metal ions such as iron in the O_2^--dependent formation of more reactive radicals is of interest because malignant disease, like chronic inflammation, produces changes in body iron distribution (Weinberg, 1981). Iron is lost from the blood (e.g. the percentage iron saturation of transferrin drops markedly) and it accumulates in liver, spleen and bone marrow. Perhaps the organism is attempting to withhold iron from the tumour and so slow its growth, since in general tumours contain less 'total iron' and have a lower degree of iron saturation in ferritin than do normal cells. This is not always the case, however, since human breast tumours appear to accumulate iron and in Hodgkin's disease heavy deposits of iron and ferritin are seen surrounding the tumour nodules (Weinberg, 1981). In some cancers, including Hodgkin's disease, breast cancer and leukaemia, the normally-low concentrations of ferritin protein present in the blood are greatly increased (e.g. Whiting *et al.*, 1981).

Abnormalities in body iron may affect the

initiation of cancer (Willson, 1977). Iron deficiency appears to predispose to oral cancers in humans and affects the pattern of tumours induced by the carcinogen 4-nitroquinoline-*N*-oxide in rats (Prime *et al.*, 1983). Several cases of primary liver cancer have been detected in iron-overloaded patients (Young & Aisen, 1982). Shires (1982) observed that incubation of rat liver nuclei with Fe(II) salts causes extensive O_2-dependent DNA damage, an observation perhaps relevant to iron overload.

Mitochondria from several malignant animal tumours are deficient in superoxide dismutase activity. These observations led Oberley & Buettner (1979) to propose that decreased activity of this enzyme together with increased radical generation might explain many of the properties of cancer cells. Many animal tumour cell lines are low in catalase activity also (Bozzi *et al.*, 1979). Growth of a transplantable tumour in mice can be decreased by injecting low molecular weight scavengers of O_2^- (Leuthauser *et al.*, 1981).

The relevance of this to human cancers is not clear, however (Michelson, 1982; Marklund *et al.*, 1982; Petkau *et al.*, 1982). In general, biopsy samples of human cancers do not seem to be deficient in superoxide dismutase or catalase activities. It should be noted that areas within a large tumour mass often have a poor blood supply and thus become anoxic. Since superoxide dismutase is induced by O_2 (Fridovich, 1978), a low activity of this enzyme could merely be a consequence of anoxia. Petkau *et al.* (1977) found that the specific activity of dismutase at the centre of a rat mammary tumour was only one-half that at the periphery, consistent with such a proposal. Rapidly-growing tumours frequently lose their histological resemblance to their tissue of origin and in evaluating changes in their superoxide dismutase activity it is important to ensure exactly which cell types are being compared, however (Oberley *et al.*, 1982). We think that present evidence does not support 'increased radical reactions' or defects in protection against radicals as a general mechanism for the origin of human cancer, although increased oxygen radical generation can lead to DNA damage and is thus potentially carcinogenic.

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